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Title: Testing for post-copulatory selection for major histocompatibility complex genotype in a semi-free-ranging primate population

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Keywords: sexual selection; reproductive success; cryptic female choice; selective fertilization; gamete choice; materno-foetal interactions; MHC; gametic union; pre-natal selection

Short title: Post-copulatory selection in mandrills

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26

ABSTRACT

A large body of evidence suggests that major histocompatibility complex (MHC) genotype influences mate choice. However, few studies have investigated MHC-mediated post-copulatory mate choice under natural, or even semi-natural, conditions. We set out to explore this question in a large semi-free-ranging population of mandrills (*Mandrillus sphinx*) using MHC-DRB genotypes for 127 parent-offspring triads. First, we showed that offspring MHC heterozygosity correlates positively with parental MHC dissimilarity suggesting that mating among MHC dissimilar mates is efficient in increasing offspring MHC diversity. Second, we compared the haplotypes of the parental dyad with those of the offspring to test whether post-copulatory sexual selection favoured offspring with two different MHC haplotypes, more diverse gamete combinations, or greater within-haplotype diversity. Limited statistical power meant that we could only detect medium or large effect sizes. Nevertheless, we found no evidence for selection for heterozygous offspring when parents share a haplotype (large effect size), genetic dissimilarity between parental haplotypes (we could detect an odds ratio of ≥ 1.86), or within-haplotype diversity (medium-large effect). These findings suggest that comparing parental and offspring haplotypes may be a useful approach to test for post-copulatory selection when matings cannot be observed, as is the case in many study systems. However, it will be extremely difficult to determine conclusively whether post-copulatory selection mechanisms for MHC genotype exist, particularly if the effect sizes are small, due to the difficulty in obtaining a sufficiently large sample.

Key words: mate choice; post-copulatory selection; gamete choice; maternal-foetal interactions; sexual selection; cryptic female choice; selective fertilization.

INTRODUCTION

The major histocompatibility complex (MHC) is one of the most polymorphic regions of the vertebrate genome [Bernatchez and Landry 2003; Piertney and Oliver 2006]. This multigene family encodes cell-surface glycoproteins that play a critical role in the immune system by recognising foreign peptides, presenting them to specialist immune cells and initiating the appropriate immune response [Klein 1986]. MHC diversity is thought to be selectively maintained, at least in part, via pathogen-mediated selection and sexual selection [Apanius et al. 1997; Piertney and Oliver 2006; Sommer 2005]. Different MHC molecules recognise and bind different foreign peptides, meaning that MHC heterozygotes should be able to present more peptides to T cells than homozygotes and thus have improved resistance to pathogens (overdominance) [Doherty and Zinkernagel 1975]. Additionally, rare MHC alleles can provide pathogen resistance when the pathogen has adapted to the majority of MHC alleles in the population (negative frequency-dependency) [Piertney and Oliver 2006].

A large body of evidence suggests that MHC genotype influences mate choice [reviews in Jordan and Bruford 1998; Penn 2002; Penn and Potts 1999; Ziegler et al. 2005]. Mate choice may occur for MHC dissimilarity between partners (disassortative mating), offering two nonexclusive fitness benefits: production of MHC heterozygous offspring [Zeh and Zeh

1997] and/or prevention of inbreeding and increase in genome-wide genetic diversity [Brown and Eklund 1994]. Alternatively, mate choice may result in selection for an optimal number of MHC alleles in the offspring [Milinski 2006], or for specific MHC genotypes, including rare alleles [Penn 2002]. The potential for MHC-mediated mate choice exists before, during and after mating [Wedekind 1994]. Evidence is available for pre-copulatory mate choice based on the MHC in rodents [Yamazaki and Beauchamp 2007], fish [Agbali et al. 2010; Consuegra and Leaniz 2008; Eizaguirre et al. 2009; Forsberg et al. 2007; Reusch et al. 2001], reptiles [Olsson et al. 2003], birds [Ekblom et al. 2004; Freeman-Gallant et al. 2003; Richardson et al. 2005], and humans [Jacob et al. 2002; Wedekind et al. 1995]. However, pre-copulatory mate choice may not always result in inheritance of a particular advantageous MHC allele for offspring because males are often heterozygous at the locus of interest, and the haploid sperm of an individual diploid male differ in their genetic compatibility with the maternal genotype [Ober 1999]. Thus, females may need post-copulatory mechanisms to ensure transmission of the desired haplotype(s) and avoid the costs of investing in a sub-optimal embryo [Wedekind 1994]. Genetic compatibility may also be detected more easily after copulation than prior to copulation, via interactions between the sperm and the female reproductive tract and ovum [Zeh and Zeh 1997].

Post-copulatory selection cannot influence which maternal MHC haplotype is passed on to offspring, as the haplotype of the ovum is set prior to fertilisation [Tulsiani 2007]. Females may, however, be able to select which paternal MHC haplotype is passed on to the offspring. Some studies have suggested that MHC molecules are expressed on the surface of spermatozooids [review in Wedekind et al. 1996], at least under certain conditions [e.g.,

infectious status, Rulicke et al. 1998]. If sperm do express their MHC haplotype, this would present an opportunity for the female reproductive tract to choose those with compatible and/or dissimilar MHC alleles, or particular alleles. In support of this possibility, *in vitro* studies have shown that gamete fusion in mice is influenced by MHC genes [Wedekind et al. 1996]. However, other studies have concluded that MHC molecules are not expressed on mature spermatozoa [e.g., Desoye et al. 1991; Hutter and Dohr 1998], making this possible mechanism of sperm choice contentious. Intriguingly, MHC-linked olfactory receptor genes are transcribed in testicular tissue, and might indirectly, signal sperm MHC haplotype via linkage disequilibrium [Ziegler et al. 2002], via an Immuno-Olfactory Supercomplex [Ziegler 1997], providing a possible alternative mechanism for MHC-associated sperm choice.

While there is considerable evidence for post-copulatory biases in fertilisation success based on overall genetic similarity in insects [Bishop 1996; Bretman et al. 2004; Mack et al. 2002; Simmons et al. 2006; Stockley 1999; Wilson et al. 1997], reptiles [Jehle et al. 2007; Olsson et al. 1996], fish [Gasparini and Pilastro 2011], and birds [Marshall et al. 2003; Thuman and Griffith 2005], relatively few studies have examined the specific role of MHC genotype in post-copulatory mate choice [but see Skarstein et al. 2005; Yeates et al. 2009 for studies in fish]. This is particularly the case for animals living and reproducing under natural, or even semi-natural, conditions, as opposed to laboratory strains, and is readily understandable as such studies must disentangle the influence of sperm competition and female effects [Birkhead 1988]. For example, male mice are sensitive to clues indicating that females have already mated and respond by allocating more sperm in each ejaculate

[Ramm and Stockley 2007], and female jungle fowl respond to the MHC similarity of a female through allocating more sperm to the more MHC-dissimilar of two females [Gillingham et al. 2009].

Selection for (or against) particular MHC combinations may also occur post-fertilisation, via selective implantation or spontaneous abortion. The survival of the fetus in the maternal environment presents an immunological paradox, as the mother must accept the presences of the equivalent of a tissue transplant [Medawar 1953], although the fetus expresses foreign (i.e., paternal) genes. Studies of maternal-fetal interactions have concentrated on spontaneous abortion in humans [Beydoun and Saftlas 2005; Makrigiannakis et al. 2011; Ober 1999]. Some studies suggest that human conceptuses inheriting paternal MHC genotypes that differ from maternal genotypes (histoincompatible pregnancies) are more likely to survive than those inheriting paternal MHC genotypes that do not differ from maternal genotypes (histocompatible pregnancies) [reviews in Beydoun and Saftlas 2005; Ober 1999], possibly because proper implantation of the embryo requires an adequate immune response. However, there is, as yet, no consensus concerning the influence of MHC allele-sharing on the risk of spontaneous abortion in humans [Beydoun and Saftlas 2005; Makrigiannakis et al. 2011], and few studies of this topic in other species.

We attempted to address the question of whether post-copulation selection occurs for MHC genotype in a population of naturally reproducing, semi-free-ranging mandrills (*Mandrillus sphinx*). We have previously shown that reproduction in this population is biased in favour of MHC-dissimilar partners [Setchell et al. 2010]. Thus far, the underlying mechanism

remains unknown, but there are theoretical reasons to expect post-copulatory selection to be common in mandrills, as in other primates [Birkhead and Kappeler 2004; Dixon 1998; Setchell and Kappeler 2003]. First, female mandrills mate with multiple males during a single fertile cycle [Setchell et al. 2005]. Second, mandrills possess very large testes relative to their body mass, suggesting high levels of sperm competition [Dixon 1998]. Finally, male coercion may limit a female's ability to express precopulatory choice; post-copulatory selection mechanisms would allow the female to overcome these constraints and favour particular males.

To test whether there is selection for or against sperm of different males, we would need to determine exactly which sperm are present in a female's reproductive tract when fertilization occurs. This requires knowledge of the exact timing of ovulation and the identity, genotype, and order of mating for all males with whom she mated during her fertile period. While the timing of ovulation can be determined using non-invasive faecal endocrinology [Hodges and Heistermann 2003], it is impossible to know the identity of all mates and the order of mating under field conditions. Moreover, the identity of the sire is likely to be influenced by sperm competition, including factors such as timing of mating relative to the optimal insemination period, ejaculate size, and position in the mating order [Birkhead and Kappeler 2004], as well as cryptic male preference for genetically dissimilar females [Gillingham et al. 2009]. We circumvented these issues by concentrating on post-copulatory selection involving the sperm of just one male – the sire. If the sire of an offspring is known, then we can be sure that his sperm were present in the mother's reproductive tract at the right time. Meiosis results in each spermatozoid being haploid and

possessing only one of the sire's two copies of each chromosome, meaning that we can test whether selection occurs between the two gametes of the same male within the female reproductive tract, based on their different genetic characteristics. Restricting analyses to the sire alone allows us to remove most of the effects of sperm competition, although meiotic drive by selfish genetic elements may result in an over-representation of one haplotype in the sperm that we cannot control for. If we detect evidence for selection *within* males, then we can extrapolate to suggest that selection will also occur *between* males.

We concentrated on MHC-DRB genes, a highly variable group of MHC class II loci that encode proteins that are directly involved in the immune response and are under strong diversifying selection pressure in mandrills, with the peptide-binding region containing significantly more non-synonymous than synonymous changes [Abbott et al. 2006]. We began by testing whether parental dyads that are MHC-dissimilar produce offspring that are more MHC diverse than offspring of less MHC-dissimilar parents (Hypothesis 1). Next, taking advantage of the fact that MHC-DRB sequences are transmitted from parent to offspring as blocks of nucleotide sequence characterized by strong linkage disequilibrium, or haplotypes, we explored which of the two haplotypes the sire contributed to each offspring, to test whether gamete selection favours MHC heterozygosity in offspring. If this is the case, then when the parents share an MHC haplotype, MHC heterozygotes (those that inherit different haplotypes from their parents) should occur more often than predicted by random inheritance, while homozygous offspring (those that inherit the same haplotype from both parents) should occur less often (Hypothesis 2).

188
189 MHC-associated selection within the reproductive tract is more likely than selection on an
190 early embryo or at the level of implantation, as it is less costly than the latter two
191 possibilities, both of which would cost a female mandrill a minimum of one reproductive
192 cycle (approx. 1 month). We tested whether gamete selection favours offspring with two
193 haplotypes that are genetically dissimilar over those with two more similar haplotypes
194 (Hypothesis 3). If this is the case, then the genetic dissimilarity between the paternal and
195 maternal haplotypes inherited by the offspring should be greater than predicted from
196 random inheritance. This differs from Hypothesis 2 because it concentrates on the genetic
197 dissimilarity *between* different MHC haplotypes, rather than presence of the same vs.
198 different haplotypes. Next, we tested whether gamete selection favours the inheritance of
199 the most diverse MHC haplotype (the haplotype possessing more MHC sequences, or MHC
200 sequences that are more functionally dissimilar) from the sire (Hypothesis 4). If this is the
201 case, then the haplotype contributed should be more diverse than the alternative haplotype
202 (i.e., it should possess more, or more functionally dissimilar, MHC sequences). This differs
203 from Hypothesis 3 by examining diversity *within* the individual haplotypes, not
204 dissimilarity *between* parental haplotypes. Finally, we examined the question of maternal-
205 fetal compatibility [Ober 1999]. If histoincompatible foetuses are more likely to survive
206 than those that are histocompatible (Hypothesis 5), then histoincompatible offspring
207 should occur more often, while histocompatible offspring should occur less often, than
208 predicted from random inheritance.

209

Despite a 20 year study, our conclusions concerning post-copulatory selection in mandrills are limited by an inability to detect small effect sizes in all analyses, and to detect even a large effect size reliably in some cases. Nevertheless, we present this study as the first exploration of gamete selection in a large primate, to propose the utility of within-sire comparisons, and as a cautionary tale in the logistical difficulties presented by such a study.

METHODS

Study population

We studied offspring born into in a large, semi-free-ranging population of mandrills at the Centre International de Recherches Médicales, Franceville (CIRMF), Gabon, over a 20 year period. The CIRMF mandrill colony was established in 1983/4, when 15 founder animals (seven males, eight females) originating from diverse locations in the wild, were released into a 6.5 ha naturally rain-forested enclosure. All further additions to the group have been due to reproduction of the founder animals and some animals have been removed. A second semi-free-ranging group was established in 1994 (3.5 ha) by transferring 17 mandrills (including four adult males and six adult females) from the first enclosure. The animals forage freely in the enclosure, and receive daily supplements of monkey chow and seasonal fruits. Water is available ad libitum. Group sizes ranged from 15 in 1983/4 to a maximum of 104 animals in 2002, similar to smaller groups observed in the wild [Rogers et al. 1996].

We assigned maternity using observations of maternal behaviour during daily observations of the colony, and subsequently confirmed these assignments using the published colony pedigree [Charpentier et al. 2005a]. The pedigree also provides an accurate paternity assignment for 193 (94 %) of the 205 offspring born [Charpentier et al. 2005a]. It was established using DNA extracted from blood samples obtained during annual captures of the colony and is based on microsatellite loci (mean loci typed per individual \pm standard error 7.42 ± 0.07). Genotypes were available for all potential sires and paternity was assigned using CERVUS 2.0 [Marshall et al. 1998] and confirmed using PARENTE [Cercueil et al. 2002] [details in Charpentier et al. 2005a].

MHC genotyping

As reported previously [Setchell et al. 2010], we genotyped 155 members of the mandrill population for MHC-DRB, including 127 offspring and their parents. Insufficient DNA was available to genotype the remaining mandrills ($N = 64$). In particular, we were unable to genotype two stillborn individuals and 18 animals that died before they could be captured. While it is possible that these animals had sub-optimal MHC genotypes (e.g., were homozygotes) and thus bias our sample towards MHC-diverse animals, many of these deaths were accidental or occurred as a result of attack by other animals, events which are likely to be independent of their MHC genotype.

The molecular methods used for MHC-DRB genotyping this mandrill population have been described previously [Abbott et al. 2006]. Briefly, we used a combination of cloning and

sequencing and denaturing gradient gel electrophoresis (DGGE) and direct sequencing to initially characterise the MHC-DRB sequences of the mandrill population. We PCR-amplified MHC-DRB sequences using the primers 5'MDRB and 3'MDRB for both procedures and the reverse primer 3'MDRB+GC for DGGE [Knapp et al, 1997]. We obtained cloned sequences in triplicate and generated DGGE sequences by removing sections of DGGE bands for reamplification via PCR followed by direct sequencing. All cloned and DGGE bands were sequenced in both directions on an ABI 373 automated sequencer (Macrogen, Korea), allowing us to eliminate artefact heteroduplex (chimeric) sequences from our genotyping results. Using these methods, we identified a total of 35 different *Mandrillus sphinx* Masp-DRB sequences. We repeated all genotyping experiments to ensure that a sequence found in one individual was also detected, if present, in relatives and all other individuals in the population. We deposited MHC sequence data in GenBank (accession numbers DQ103715–DQ103732, DQ103734–DQ103746, EU693911–EU693914).

We used two methods to differentiate functional MHC-DRB genes from nonfunctional pseudogenes. First, we reviewed all sequences for stop codons [Abbott et al, 2006]. One sequence (*Masp*-DRB-6*0404) had a stop codon, so we removed this from the dataset for analysis. Next, we examined patterns of transcription using cDNA from a subset of seven mandrills representing all known *Masp*-DRB loci and lineages, and for whom mRNA samples were available [Setchell et al. 2010]. We found that 15 / 16 of the mandrill MHC-DRB sequences identified in these animals were transcribed and, therefore, possibly functional (although we did not investigate whether the sequences were translated). The one sequence that was undetected using cDNA (*Masp*-DRB 6*0402) had a 1 bp deletion,

279 which would disrupt the sequence reading frame and render it incapable of making a
280 functional protein. Therefore, we also removed this sequence from our analyses. Human
281 MHC-DRB6 sequences, traditionally characterised as pseudogenes due to mutations in exon
282 2, may only exhibit low levels of expression [Fernandez-Soria et al., 1998] so it is
283 unsurprising that these two mandrill DRB6 sequences would be nonfunctional.
284 Transcription of other *Masp*-DRB6 sequences, was uncertain, as we were unable to obtain
285 mRNA for cDNA analyses, but these sequences had no stop codons or nonsense mutations
286 to render them obviously nonfunctional. One of these, *Masp*-DRB6*0401, was found in a
287 fairly large number of individuals (10 % of the population), and 10 of the offspring
288 analysed (8 %). Two other *Masp*-DRB6 sequences were present in only eight (*Masp*-
289 DRB6*0102) and one (*Masp*-DRB6*0101) individuals. Removing these individuals from the
290 analysis did not alter our conclusions.

291
292 The MHC-DRB region in Old World primates frequently experiences expansion through
293 gene duplication and contraction through deletion [Slierendregt et al. 1994]. Because of the
294 extensive variation in DRB haplotype composition, individuals possess different numbers
295 and types of DRB genes on each haplotype. We focused on these haplotypes, without
296 making any assumptions about the number of loci involved. We deduced haplotypes
297 (unique combinations of sequences inherited together from parent to offspring) via
298 patterns of inheritance using known parent-offspring triads from the colony pedigree. For
299 example, female 2's first offspring (mandrill 2A) was sired by male 7. The MHC genotypes
300 of the triad are shown in Table 1. In this case, we can see that offspring 2A shares both
301 *W7001 and *W7101 with female 2, but male 7 does not have these sequences, so we can

deduce that 2A must have inherited both from female 2. Thus 2A's maternal haplotype consists of sequences *W7001 and *W7101. Similarly, 2A shares only sequence 1*0404 with male 7. As female 2 does not possess this sequence, 2A must have inherited it from male 7. Thus 2A's paternal haplotype consists of only the sequence 1*0404. Further, sequences not passed to 2A by female 2, which must therefore be in her other, non-transmitted, haplotype, were 1*0302, 5*0302, and sequences not passed on to offspring 2A by male 7, the non-transmitted paternal haplotype, were 3*0402 and *W401.

We proceeded in a similar way for all offspring, identifying a total of 17 different MHC haplotypes in the 155 animals genotyped. Each haplotype consisted of 1-4 sequences (mean 2.4), and was present in 1-3 of the founder individuals (mean \pm SEM = 1.5 \pm 0.2) and 1-75 of all individuals (mean \pm SEM = 17.1 \pm 4.3). Each individual mandrill possessed 1-7 sequences, in two haplotypes; when we found only one haplotype in an individual we assumed the individual was homozygous for that haplotype. We detected no changes in MHC haplotype from parent to offspring in our dataset, suggesting that no major recombination occurred in our sample.

We examined MHC sequence diversity in terms of the number of sequences in each haplotype. As MHC sequences may differ in nucleotide composition, but still share the same amino acid sequences due to the presence of silent substitutions, we calculated the number of amino acid differences between each pair of MHC sequences as an estimate of genetic dissimilarity [Landry et al. 2001]. Additionally, since not all amino acids are involved in peptide binding, we also calculated the number of amino-acid differences in the predicted

325 peptide binding region (PBR, based on the PBR for human sequences) between each pair of
326 MHC sequences as an estimate of genetic dissimilarity.

327

328 We calculated three measures of MHC-dissimilarity between the mother's haplotype and
329 each of the sire's haplotypes:

330 MHC_{diff} The total number of different MHC sequences in the two haplotypes.

331 AA_{diff} The sum of all pairwise amino acid differences between the sequences of the
332 two haplotypes.

333 PBR_{diff} The sum of all pairwise amino acid differences between the peptide binding
334 sites of the two haplotypes.

335

336 We described the within-haplotype diversity of the two available MHC haplotypes for each
337 parent as follows:

338 MHC_n The number of MHC sequences in the haplotype.

339 AA_n The sum of all pairwise amino acid differences between all sequences on the
340 haplotype.

341 PBR_n The sum of all pairwise amino acid differences between the peptide binding
342 sites of the sequences on the haplotype.

343

344 ***Statistical analyses***

345

346 We used a mixed model (in SPSS) including dyad identity as a random effect to compare
347 parental MHC-dissimilarity (measured as the total number of different MHC sequences

possessed by a mother x sire dyad) with the number of MHC sequences in the offspring (Hypothesis 1).

To test whether selection resulted in more heterozygous offspring than expected by chance (Hypothesis 2), we compared the inheritance patterns of paternal haplotypes, given the known maternal haplotype, with the 50:50 expected from chance using binomial tests.

To test whether selection occurred for more dissimilar combinations of haplotypes over similar combinations (Hypothesis 3) we compared MHC_{diff} and AA_{diff} for the maternal haplotype with each of the two alternative paternal haplotypes and tested for differences in similarity using Wilcoxon matched-paired tests. We also used Wilcoxon paired tests to test for selection for more MHC diverse paternal haplotypes, irrespective of the female haplotype (Hypothesis 4), by comparing MHC_n and AA_n in paternal haplotypes that were transmitted with those that were not.

Finally, to test whether an excess of histoincompatible offspring was born (Hypothesis 5), we split possible conceptuses into the following categories, following Ober (1999), and compared observed offspring with those predicted from random inheritance of haplotypes using a chi-squared test:

- Likely to be histoincompatible: paternally inherited allele different from both maternal alleles. (As there is very little information about acceptable or unacceptable MHC-DRB mismatches in Old World monkeys, it may be that not all non-identical MHC-DRB molecules are ‘histoincomaptible’).

- Homozygous histocompatible: paternally inherited allele is the same as maternally inherited allele.
- Heterozygous histocompatible: paternally inherited allele matches the maternal allele that was not inherited.

We used G*Power 3 [Faul et al. 2007] to determine the statistical power of our analyses.

We used *sensitivity analyses* to compute the critical population effect size as a function of α (set as 0.05), $1 - \beta$ (set as 0.90) and N (the sample size) and *a priori* power analyses to determine the sample size necessary to detect Cohen's standardised *small, medium* and *large* effect size conventions [Cohen 1988] for each analysis. In the case of the MDC procedure we calculated power using a standard logistic regression.

We focussed our analyses at the level of the offspring. However, mothers ($N = 31$, range: 1-11, mean 4.1), sires ($N = 15$, range: 2-30, mean = 8.0), and mother-sire dyads ($N = 75$, range: 1-6, mean = 1.6) each contributed multiple offspring to the dataset, leading to the potential for pseudo-replication, if individuals or dyads show biased MHC transmission. With the exception of the initial mixed model, we were unable to control for this, as it was not possible to include parent or dyadic ID as a random factor in our analyses.

Unfortunately, the relatively low numbers of offspring contributed by individual animals and the diversity of MHC haplotypes in the population meant that the occurrence of any particular MHC haplotype was too low to test for the transmission of particular MHC haplotypes with reasonable statistical power.

Ethics statement

This research complied with protocols approved by the Comité Régional d'Ethique Ile de France Paris Sud (registration number 02-010) and adhered to the legal requirements of the country in which the research was conducted (Gabon). The research adhered to the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non-Human Primates. The CIRMF mandrills are housed in very large, naturally rain-forested enclosures, where they forage naturally and receive twice-daily provisioning. Animals remained in the enclosures during and after the study. The only invasive procedure involved was blood sampling for DNA, undertaken during routine annual veterinary controls of the mandrill colony, during which all efforts were made to ameliorate suffering.

RESULTS

MHC-dissimilar parents produce MHC-diverse offspring

Parents that were more MHC-dissimilar had offspring with a greater number of different MHC sequences than offspring from MHC-similar dyads (mixed model with dyad identity as a random effect: $F = 28.04$, d.f. = 1, 66, $P < 0.001$, Fig. 1).

Is there selection for heterozygous offspring when parents share a haplotype?

Of 18 cases where one of the two possible paternal haplotypes was the same as the

maternal haplotype, that paternal haplotype was passed on in 11 cases, which did not differ significantly from chance (one-tailed binomial exact test: $P = 0.240$). However, to detect even a large effect size (0.25) in this analysis would require a sample size of 33 offspring, so this may reflect Type 2 error (i.e., failure of the test to detect a real relationship). Thus, we cannot conclude that there is no selection for heterozygous offspring when parents share a haplotype.

Is there selection for more MHC dissimilar parental haplotypes?

We found no influence of genetic dissimilarity on whether a paternal haplotype was inherited (Table 2). With our sample size ($N = 127$), we would be able to detect an effect size of 0.267 (i.e., between a small (0.2) and medium (0.5) effect size). Detection of a small effect would require a sample size of 226. Thus we can conclude that there is no medium-large effect, but we are unable to rule out a small-medium effect of selection for more dissimilar haplotypes.

Is there selection for transmission of more diverse haplotypes?

Within-haplotype MHC diversity was not greater in the paternal haplotype that was passed on to the offspring than in the one that was not (Table 2). As above, we can conclude that there is no medium-large effect, but we are unable to rule out a small-medium effect of selection for more diverse haplotypes.

440 ***Is there selection for maternal-fetal MHC histoincompatibility?***

441
442 The 96 offspring born to parents that shared no haplotypes (born to 51 dyads composed of
443 27 mothers and 14 sires) were likely to be histoincompatible. In the 31 cases where
444 parents of an offspring shared an MHC haplotype (20 dyads composed of 14 mothers and
445 10 sires), they produced a heterozygous histocompatible offspring 6 times, a homozygous
446 histocompatible offspring 11 times and a histoincompatible offspring 14 times. This
447 distribution does not differ from chance ($\chi^2 = 1.903$, $df = 2$, $P = 0.386$). However, to detect
448 even a large effect (0.5) using this test would require a sample size of 51. Lumping
449 heterozygous and homozygous histocompatible offspring did not improve this situation as
450 the threshold to detect a large effect size in this comparison is 43.

451
452 **DISCUSSION**

453
454 We attempted to address an intriguing question in evolutionary biology - whether MHC-
455 dependent post-copulatory mate choice occurs - in a large primate species. First, we
456 showed that offspring MHC heterozygosity correlates positively with parental MHC
457 dissimilarity in our study population. This is not surprising and shows that mating among
458 MHC dissimilar parents, which is known to occur in mandrills [Setchell et al. 2010], is
459 efficient in increasing offspring MHC diversity. Similar findings have been reported for
460 white-toothed shrews, *Crocidura russula* [Duarte et al. 2003]; Seychelles warblers,
461 *Acrocephalus sechellensis* [Richardson et al. 2004]; and house finches, *Carpodacus*
462 *mexicanus* [Oh and Badyaev 2006].

463

464 Next, we attempted to test whether there is gamete selection for MHC heterozygosity,
465 dissimilarity between parental haplotypes, or within-haplotype diversity. We genotyped
466 127 offspring born over 20 years, circumventing problems associated with differential
467 sperm allocation by males by concentrating on within-sire haplotype selection. Problems
468 with statistical power plague animal behaviour research [Smith et al. 2011], and it is often
469 very difficult to increase the sample size [Taborsky 2010]. Our study is no exception, and
470 our conclusions are limited by an inability to detect small effects in all analyses, and to
471 detect even a large effect of maternal-fetal histocompatibility.

472

473 The lack of immigration into the CIRMF colony means that the potential for inbreeding has
474 increased with subsequent generations. Previous studies of the colony have demonstrated
475 the effects of inbreeding on fitness correlates: female body mass and size decrease with
476 inbreeding, as does age at first birth, which may be an indirect consequence of the effect of
477 inbreeding on body mass and size [Charpentier et al. 2006]. Moreover, there is a positive
478 relationship between genetic diversity and reproductive success in both males and females
479 [Charpentier et al. 2005b], and a positive relationship between MHC diversity and
480 reproductive success in males [Setchell et al. 2010]. Theoretically, a risk of inbreeding
481 should, if anything, lead to increased selection in favour of genetic diversity. For example,
482 studies of inbred laboratory strains of rats [Michie and Anderson 1966; Palm 1969] and
483 mice [Hamilton and Hellstrom 1978], where new-borns show a deficit of MHC
484 homozygotes and increased heterozygosity. However, even in this closed colony, which
485 results from a small number of founders, with no immigration, only a minority of offspring

(31/127) were born to parents that share MHC haplotypes, limiting our potential to examine questions of heterozygote excess and materno-fetal compatibility in particular. Nevertheless, we found no evidence for a large post-copulatory selection effect in favour of offspring with two different MHC haplotypes where the parents shared a haplotype, at least within males, although we did not have the power to detect medium or small effect sizes. We also found no evidence for a medium-large effect of selection for more dissimilar parental haplotypes, or for selection for more within-haplotype diversity, although we did not have the power to detect small effects.

Reproduction in the CIRMF mandrills is biased in favour of genetically dissimilar dyads and MHC-diverse males [Setchell et al. 2010]. The sexual dimorphism found in mandrills, and the polygynandrous nature of their mating system suggests that this may be due to post-copulatory selection, at least in part. However, we found no evidence of a medium-large effect of MHC-associated post-copulatory selection, although our sample size was too small to detect any small-medium effect. Possible MHC-associated post-copulatory selection has been found in mouse lemurs (*Microcebus murinus*), in which a study comparing sires and non-sires (i.e., *between* male comparisons) of 79 offspring found no evidence for pre-copulatory female choice based on male MHC genotype. However, sires differed significantly at the MHC from randomly assigned males, possessing fewer MHC sequences different to those of the female, but a higher number of MHC supertype differences different to those of the female, as well as fewer MHC sequences but more superotypes overall [Schwensow et al. 2008]. Intriguingly, studies of fish have provided contrasting results: MHC-heterozygous males had higher fertilisation success than MHC-homozygotes

in charr (*Salvelinus alpinus*) [Skarstein et al. 2005], whereas male Atlantic salmon (*Salmo salar*) obtained greater relative fertilization success when competing for eggs from MHC-similar females, a finding possibly related to the importance of avoiding outbreeding in this species [Yeates et al. 2009]. Together, these results suggest that post-copulatory selection for MHC can occur, at least *between* males, although the patterns observed differ between species. In a *within* male comparison in sedge warblers (*Acrocephalus schoenobaenus*), offspring had a higher overall genetic diversity (based on microsatellite genotype) than expected if fertilisation was random [Marshall et al. 2003], with a medium-large effect size (calculated as $Z / \sqrt{N} = 0.45$), suggesting that selective fertilisation can occur within males, at least in birds. Our statistical power was sufficient to detect an effect of similar size in mandrills, but we did not find one.

Particular MHC haplotypes may be preferentially transmitted due to a selective advantage associated with the non-transmitted haplotype, for example in relation to specific parasites [review in Piertney and Oliver 2006]. Particular MHC haplotypes occurred at too low a rate in our study population to test for the transmission of particular MHC haplotypes with reasonable statistical power. However, Milinski (2006) has argued that females are not only unlikely to be able to detect the presence of individual MHC alleles, but they are probably also unlikely to know the precise relationship between specific parasites and MHC alleles, suggesting that mate selection for specific alleles is unlikely to occur.

Alternatively, as noted for humans [Diamond 1987], certain MHC alleles may be linked to other genetic loci that have their own advantages or disadvantages and this linkage disequilibrium may result in biased transmission of the linked loci.

532

533 While we are unable to determine conclusively whether mandrills employ gamete selection
534 for MHC diversity, a non-exclusive alternative mechanism underlying preferential
535 reproduction with MHC-dissimilar mates [Setchell et al. 2010] relies on chemical
536 communication. Both male and female mandrills possess a sternal gland, which produces a
537 glandular secretion, which may play a role in the pre-copulatory assessment of MHC
538 compatibility via 'fragrant genes' [Milinski 2006]. In support of this hypothesis, we have
539 recently shown that odour similarity reflects similarity at the MHC in our study population,
540 suggesting that odour provides information against which the receiver can compare its
541 own genotype to assess genetic similarity [Setchell et al. 2011]. Without additional post-
542 copulatory processes, pre-copulatory selection based on odour cannot select for particular
543 haplotypes, as an individual transmits either of its two haplotypes. If MHC-associated mate
544 choice does occur pre-copulation, then this may imply that the specific MHC Class II
545 haplotype passed on by a chosen partner is less important than overall genetic
546 dissimilarity, and that MHC-DRB diversity may be maintained as a consequence of selection
547 for overall genetic dissimilarity [Brown and Eklund 1994], rather than selection for MHC
548 diversity itself. However, our previous results suggest that the influence of MHC
549 dissimilarity on reproduction was stronger than that of overall genetic dissimilarity
550 [Setchell et al. 2010].

551

552 In conclusion, we set out to test for MHC-mediated post-copulatory selection in mandrills
553 by genotyping as many of the 205 offspring born into the CIRMF mandrill colony over 20
554 years as possible (127). However, this sample size gave us the possibility of detecting

medium effect sizes at best, and in some cases we were unable to detect even a large effect size. With these limitations, we found no evidence for large effect sizes of MHC-mediated post-copulatory selection in this species. Our concentration on comparing the haplotypes found in the sire and the mother with those of the resulting offspring may represent a way forward in future, large-scale studies of the genetics of natural and semi-natural populations, providing a window onto potential post-copulatory selection mechanisms. Females of many primate species, including mandrills, mate with multiple males [Dixson 1998], and these are the species in which post-copulatory selection should be expected [Setchell and Kappeler 2003]. However, field-workers are unlikely to be able to document all mating events reliably, with the exception of species that experience a very short receptive period [e.g., one night in mouse lemurs, Schwensow et al. 2008]. If gamete selection occurs at the level of the individual sperm, then it should detect selection *between* sperm of the same male. Any selection for particular characteristics of the sperm, resulting from selection in the female reproductive tract, egg choice or selection following conception in the oviduct would be detectable in such an analysis. While we cannot pinpoint the exact timing of any such selection events, we suspect that any such selection would occur relatively early, via sperm selection within the female reproductive tract or egg choice for particular fertilising sperm. Later selection, for example selection on the early embryo in oviduct, implantation, spontaneous abortion, and pre- and post-natal investment [Ober 1999], would all incur relatively high costs for female primates, since selection post-fertilisation would involve a delay in pregnancy of at least one menstrual cycle, and possibly result in birth during a sub-optimal period in seasonal breeders.

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REFERENCES CITED

- Abbott KA, Wickings EJ, Knapp LA. 2006. High levels of diversity characterize mandrill (*Mandrillus sphinx*) Mhc-DRB sequences. Immunogenetics 58:628-640.
- Agbali M, Reichard M, Bryjová A, Bryja J, Smith C. 2010. Mate choice for nonadditive genetic benefits correlate with mhc dissimilarity in the rose bitterling (*Rhodeus ocellatus*). Evolution 64:1683 - 1696.
- Apanius V, Penn D, Slev PR, Ruff LR, Potts WK. 1997. The nature of selection on the major histocompatibility complex. Crit Rev Immunol 17:179-224.
- Bernatchez L, Landry C. 2003. MHC studies in nonmodel vertebrates: what have we learned about natural selection in 15 years? J Evol Biol 16:363-377.
- Beydoun H, Saftlas AF. 2005. Association of human leukocyte antigen sharing with recurrent spontaneous abortions. Tissue Antigens 65:123-135.

601 Birkhead. 1988. Cryptic female choice: criteria for establishing female sperm choice.
602 Evolution 52:1212-1218.

603 Birkhead TR, Kappeler PM. 2004. Post-copulatory sexual selection in birds and primates.
604 In: Kappeler PM, van Schaik CP, editors. Sexual Selection in Primates: New and
605 Comparative Perspectives. Cambridge: Cambridge University Press. p 151-171.

606 Bishop JDD. 1996. Female control of paternity in the internally fertilizing compound
607 ascidian *Diplosoma listerianum*. I. Autoradiographic investigation of sperm
608 movements in the female reproductive tract. Proc Roy Soc Lond B 263:369–376.

609 Bretman A, Wedell N, Tregenza T. 2004. Molecular evidence of post-copulatory inbreeding
610 avoidance in the field cricket *Gryllus bimaculatus*. Proc Roy Soc Lond B 271:159–
611 164.

612 Brown JL, Eklund A. 1994. Kin recognition and the major histocompatibility complex: an
613 integrative review. Am Nat 143:435–461.

614 Cercueil A, Bellemain E, Manel S. 2002. Parente: a software package for parentage analysis.
615 J Hered 93:458-459.

616 Charpentier M, Peignot P, Hossaert-McKey M, Gimenez O, Setchell JM, Wickings EJ. 2005a.
617 Constraints on control: Factors influencing reproductive success in male mandrills
618 (*Mandrillus sphinx*). Behav Ecol 16:614-623.

619 Charpentier M, Setchell JM, Prugnolle F, Knapp LA, Wickings EJ, Peignot P, Hossaert-McKey
620 M. 2005b. Genetic diversity and reproductive success in mandrills (*Mandrillus*
621 *sphinx*). Proc Natl Acad Sci USA 102:16723-16728.

622 Charpentier M, Setchell JM, Prugnolle F, Wickings EJ, Peignot P, Balloux F, Hossaert-McKey
623 M. 2006. Life history correlates of inbreeding depression in mandrills (*Mandrillus*
624 *sphinx*). Mol Ecol 15:21-28.

625 Cohen J. 1988. Statistical Power Analysis for the Behavioral Sciences. New Jersey: Lawrence
626 Erlbaum Ass. 590 p.

627 Consuegra S, Leaniz CGd. 2008. MHC-mediated mate choice increases parasite resistance in
628 salmon. Proc Roy Soc Lond B 275:1397-1403.

629 Desoye G, Dohr GA, Ziegler A. 1991. Expression of human major histocompatibility antigens
630 on germ cells and early preimplantation embryos. Lab Invest 64:306-312.

631 Diamond JM. 1987. Causes of death before birth. Nature 329:487-488.

632 Dixson AF. 1998. Primate Sexuality: Comparative Studies of the Prosimians, Monkeys, Apes
633 and Human Beings. Oxford: Oxford University Press. 560 p.

634 Doherty P, Zinkernagel R. 1975. Enhanced immunological surveillance in mice
635 heterozygous at the H-2 gene complex. Nature 256:50-52.

636 Duarte L, Bouteiller C, Fontanillas I, Petit E, Perrin N. 2003. Inbreeding in the greater white-
637 toothed shrew, *Crocidura russula*. Evolution 57:638-45.

638 Eizaguirre C, S. E. Yeates, T. L. Lenz, M. Kalbe, Milinski M. 2009. MHC-based mate choice
639 combines good genes and maintenance of MHC polymorphism. Mol Ecol 18:3316-
640 3329.

641 Ekblom R, Saether SA, Grahn M, Fiske P, Kalas JA, Hoglund J. 2004. Major histocompatibility
642 complex variation and mate choice in a lekking bird, the great snipe (*Gallinago*
643 *media*). Mol Ecol 13:3821-3828.

644 Faul F, Erdfelder E, Lang A-G, Buchner A. 2007. G*Power 3: A flexible statistical power
645 analysis program for the social, behavioral, and biomedical sciences. Behav Res
646 Meth 39:175-191.

647 Fernandez-Soria VM, Morales P, Castro MJ, Suarez B, Recio MJ, Moreno MA, Paz-Artal E,
648 Arnaiz-Villena A. 1998. Transcription and weak expression of HLA-DRB6: a gene
649 with anomalies in exon 1 and other regions. Immunogenetics 48:16-21.

650 Forsberg LA, Dannewitz J, Petersson E, Grahm M. 2007. Influence of genetic dissimilarity in
651 the reproductive success and mate choice of brown trout - females fishing for
652 optimal MHC dissimilarity. J Evol Biol 20:1859–1869.

653 Freeman-Gallant CR, Meguerdichian M, Wheelwright NT, Sollecito SV. 2003. Social pairing
654 and female mating fidelity predicted by restriction fragment length polymorphism
655 similarity at the major histocompatibility complex in a songbird. Mol Ecol 12:3077 -
656 3083.

657 Gasparini C, Pilastro A. 2011. Cryptic female preference for genetically unrelated males is
658 mediated by ovarian fluid in the guppy. Proc Roy Soc Lond B 278:2495-2501.

659 Gillingham MAF, Richardson DS, L'Abbe H, Moynihan A, Worley K, Pizzari T. 2009. Cryptic
660 preference for MHC-dissimilar females in male red junglefowl, *Gallus gallus*. Proc
661 Roy Soc Lond B 276:1083-1092.

662 Hamilton MS, Hellstrom I. 1978. Selection for histoincompatible progeny in mice. Biol
663 Reprod 19:267-70.

664 Hodges JK, Heistermann M. 2003. Field endocrinology: monitoring hormonal changes in
665 free-ranging primates. In: Setchell JM, Curtis DJ, editors. Field and Laboratory
666 Methods in Primatology: A Practical Guide. Cambridge: Cambridge University Press.

667 Hutter H, Dohr G. 1998. HLA expression on immature and mature human germ cells. J
 668 Reprod Immunol 38:101–122.

669 Jacob S, McClintock MK, Zelano B, Ober C. 2002. Paternally inherited HLA alleles are
 670 associated with women's choice of male odor. Nature Genetics 30:175-179.

671 Jehle R, Szatcensny M, Wolf JBW, Whitlock A, Hoedl W, Burke T. 2007. Genetic dissimilarity
 672 predicts paternity in the smooth newt (*Lissotriton vulgaris*). Biol Lett 3:526-528.

673 Jordan WC, Bruford MW. 1998. New perspectives on mate choice and the MHC. Heredity
 674 81:127-133.

675 Klein J. 1986. The Natural History of the Major Histocompatibility Complex. New York:
 676 Wiley. 775 p.

677 Knapp LA, Cadavid LF, Eberle ME, Knechtle SJ, Bontrop RE, Watkins DI. 1997. Identification
 678 of new Mamu-DRB alleles using DGGE and direct sequencing. Immunogenetics
 679 45:171–179.

680 Landry C, Garant D, Duchesne P, Bernatchez L. 2001. 'Good genes as heterozygosity': the
 681 major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*).
 682 Proc Roy Soc Lond B 268:1279-1285.

683 Mack PD, Hammock BA, Promislow DEL. 2002. Sperm competitive ability and genetic
 684 relatedness in *Drosophila melanogaster*: similarity breeds contempt. Evolution
 685 56:1789–1795.

686 Makrigiannakis A, Petsas G, Toth B, Relakis K, Jeschke U. 2011. Recent advances in
 687 understanding immunology of reproductive failure. Journal of Reproductive
 688 Immunology 90:96-104.

689 Marshall RC, Buchanan KL, Catchpole CK. 2003. Sexual selection and individual genetic
690 diversity in a songbird. *Proc Roy Soc Lond B* 270:S248-S250.

691 Marshall TC, Slate J, Kruuk LEB, Pemberton JM. 1998. Statistical confidence for likelihood-
692 based paternity inference in natural populations. *Mol Ecol* 7:639-655.

693 Medawar P. 1953. Some immunological and endocrinological problems raised by evolution
694 of viviparity in vertebrates. *Symp Soc Exp Biol* 7:320-328.

695 Michie D, Anderson NF. 1966. A strong selective effect associated with a histocompatibility
696 gene in the rat. *Ann New York Acad Sci* 129:88-93.

697 Milinski M. 2006. The major histocompatibility complex, sexual selection, and mate choice.
698 *Ann Rev Ecol Evol System* 37:159-186.

699 Ober C. 1999. Studies of HLA, fertility and mate choice in a human isolate. *Hum Reprod*
700 *Update* 5:103-107.

701 Oh KP, Badyaev AV. 2006. Adaptive genetic complementarity in mate choice coexists with
702 selection for elaborate sexual traits. *Proc Roy Soc Lond B* 273:1913 - 1919.

703 Olsson M, Madsen T, Nordby J, Wapstra E, Ujvari B, Wittsell H. 2003. Major
704 histocompatibility complex and mate choice in sand lizards. *Proc Roy Soc Lond B*
705 270:S254-S256.

706 Olsson M, Shine R, Madsen T, Gullberg A, Tegelstrom H. 1996. Sperm selection by females.
707 *Nature* 383:585.

708 Palm J. 1969. Association of maternal genotype and excess heterozygosity for Ag-B
709 histocompatibility antigens among male rats. *Transplant Proc* 1:82-84.

710 Penn DJ. 2002. The scent of genetic compatibility: sexual selection and the major
711 histocompatibility complex. *Ethology* 108:1-21.

712 Penn DJ, Potts WK. 1999. The evolution of mating preferences and major histocompatibility
 713 complex genes. *Am Nat* 153:145-164.

714 Piertney SB, Oliver MK. 2006. The evolutionary ecology of the major histocompatibility
 715 complex. *Heredity* 96:7-21.

716 Ramm SA, Stockley P. 2007. Ejaculate allocation under varying sperm competition risk in
 717 the house mouse, *Mus musculus domesticus*. *Behav Ecol* 18:491-495.

718 Reusch TBH, Haberli MA, Aeschlimann PB, Milinski M. 2001. Female sticklebacks count
 719 alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature*
 720 414:300-302.

721 Richardson DS, Komdeur J, Burke T, Bjarklund M. 2004. Inbreeding in the Seychelles
 722 warbler: environment-dependent maternal effects. *Evolution* 58:2037-2048.

723 Richardson DS, Komdeur J, Burke T, von Schantz T. 2005. MHC-based patterns of social and
 724 extra-pair mate choice in the Seychelles warbler. *Proc Roy Soc Lond B* 272:759 -
 725 767.

726 Rogers ME, Abernethy KA, Fontaine B, Wickings EJ, White LJT, Tutin CEG. 1996. Ten days in
 727 the life of a mandrill horde in the Lope Reserve, Gabon. *Am J Primatol* 40:297-313.

728 Rulicke T, Chapuisat M, Homberger FR, Macas E, Wedekind C. 1998. MHC-genotype of
 729 progeny influenced by parental infection. *Proc Roy Soc Lond B* 265:711-716.

730 Schwensow N, Eberle M, Sommer S. 2008. Compatibility counts: MHC-associated mate
 731 choice in a wild promiscuous primate. *Proc Roy Soc Lond B* 275:555-564.

732 Setchell J, Vaglio S, Abbott KM, Moggi-Cecchi J, Boscaro F, Pieraccini G, Knapp LA. 2011.
 733 Odour signals MHC genotype in an Old World monkey. *Proc Roy Soc Lond B*
 734 278:274-280.

735 Setchell JM, Charpentier M, Wickings EJ. 2005. Mate-guarding and paternity in mandrills
736 (*Mandrillus sphinx*): Factors influencing monopolisation of females by the alpha
737 male. Anim Behav 70:1105-1120.

738 Setchell JM, Charpentier MJE, Abbott KA, Wickings EJ, Knapp LA. 2010. Opposites attract:
739 MHC-associated mate choice in an anthropoid primate. J Evol Biol 23:136-148.

740 Setchell JM, Kappeler PM. 2003. Selection in relation to sex in primates. Adv Stud Behav
741 33:87-173.

742 Simmons LW, Beveridge M, Wedell N, Tregenza T. 2006. Postcopulatory inbreeding
743 avoidance by female crickets only revealed by molecular markers. Mol Ecol
744 15:3817-3824.

745 Skarstein F, Folstad I, Liljedal S, Grahm M. 2005. MHC and fertilization success in the Arctic
746 charr (*Salvelinus alpinus*). Behav Ecol Sociobiol 57:374-380.

747 Slierendregt BL, Otting N, van Besouw N, Jonker M, Bontrop RE. 1994. Expansion and
748 contraction of rhesus macaque DRB regions by duplication and deletion. Journal of
749 Immunology 152:2298-2307.

750 Smith DR, Hardy ICW, Gammell MP. 2011. Power rangers: no improvement in the statistical
751 power of analyses published in Animal Behaviour. Anim Behav 81:347-352.

752 Sommer S. 2005. The importance of immune gene variability (MHC) in evolutionary
753 ecology and conservation. Frontiers in Zoology 2:16.

754 Stockley P. 1999. Sperm selection and genetic incompatibility: does relatedness of mates
755 affect male success in sperm competition? Proc Roy Soc Lond B 266:1663-1669.

756 Taborsky M. 2010. Sample size in the study of behaviour. Ethology 116:185-202.

757 Thuman KA, Griffith SC. 2005. Genetic similarity and the nonrandom distribution of
 758 paternity in a genetically highly polyandrous shorebird. *Anim Behav* 69:765–770.
 759 Tulsiani D. 2007. *Introduction to Mammalian Reproduction*. New York: Springer-Verlag.
 760 Wedekind C. 1994. Mate choice and maternal selection for specific parasite resistances
 761 before, during and after fertilization. *Phil Trans R Soc Lond B* 346:303-311.
 762 Wedekind C, Chapuisat M, Macas E, Rulicke T. 1996. Nonrandom fertilization in mice
 763 correlates with the MHC and something else. *Heredity* 77:400–409.
 764 Wedekind C, Seebeck T, Bettens F, J. PA. 1995. MHC-dependent mate preferences in
 765 humans. *Proc Roy Soc Lond B* 260:245-249.
 766 Wilson N, Tubman SC, Eady PE, Robertson GW. 1997. Female genotype affects male success
 767 in sperm competition. *Proc Roy Soc Lond B* 1387:1491-1495.
 768 Yamazaki K, Beauchamp G. 2007. Genetic basis for MHC-dependent mate choice. *Adv Gen*
 769 59:130-145.
 770 Yeates SE, Einum S, Fleming IA, Megens H-J, Stet RJM, Hindar K, Holt WV, Van Look KJW,
 771 Gage MJG. 2009. Atlantic salmon eggs favour sperm in competition that have similar
 772 major histocompatibility alleles. *Proc Roy Soc Lond B* 276:559-566.
 773 Zeh JA, Zeh DW. 1997. The evolution of polyandry. II. Post-copulatory defences against
 774 genetic incompatibility. *Proc Roy Soc Lond B* 264:69-75.
 775 Ziegler A. 1997. Biology of chromosome 6. *DNA Sequence* 8:189–202.
 776 Ziegler A, Dohr G, Uchanska-Ziegler B. 2002. Possible roles for products of polymorphic
 777 MHC and linked olfactory receptor genes during selection processes in
 778 reproduction. *Am J Reprod Immunol* 48:34–42.

779 Ziegler A, Kentenich H, Uchanska-Ziegler B. 2005. Female choice and the MHC. Trends
780 Immunol 26:496-502.
781

Table 1: MHC-DRB genotypes of one parent-offspring triad from the CIRMF mandrill population. M indicates a maternal haplotype, S a paternal haplotype. Sequences not present in these three individuals are not shown, for simplicity

	MHC-DRB sequences possessed						
	1*0302	1*0404	3*0402	5*0302	*W401	*W7001	*W7101
Mother (female 2)	M1			M1		M2	M2
Sire (male 7)		S1	S2		S2		
Offspring (2A)		S1				M2	M2

Table 2: Comparison of MHC diversity in paternal haplotypes inherited by offspring and those that were not (results of Wilcoxon paired tests, N = 127)

MHC variable ^a	Haplotype inherited (mean +/- SE)	Haplotype not inherited (mean +/- SE)	Z	P
<i>MHC_{diff}</i>	3.7 +/- 0.1	3.9 +/- 0.1	0.977	0.328
<i>AA_{diff}</i>	55.9 +/- 4.0	52.9 +/- 3.3	0.009	0.993
<i>PBR_{diff}</i>	33.4 +/- 1.8	32.7 +/- 1.6	0.215	0.830
<i>MHC_n</i>	1.9 +/- 0.1	2.0 +/- 0.1	0.447	0.655
<i>AA_n</i>	18.1 +/- 1.9	17.3 +/- 1.8	0.135	0.893
<i>PBR_n</i>	14.3 +/- 1.1	13.1 +/- 1.0	0.751	0.453

^a *MHC_{diff}*: the number of different MHC sequences in the two haplotypes; *AA_{diff}*: the sum of all pairwise amino acid differences between the sequences of the two haplotypes; *PBR_{diff}*: the sum of all pairwise amino acid differences between the peptide binding sites of the two haplotypes; *MHC_n*: the number of MHC sequences in the haplotype; *AA_n*: the sum of all pairwise amino acid differences between all sequences on the haplotype; *PBR_n*: the sum of all pairwise amino acid differences between the peptide binding sites of the sequences on the haplotype.

Figure 1: Comparison of the number of MHC sequences in offspring with the number of different MHC sequences in the parents. Point size indicates number of overlapping data points.

